

CLAIMS

- 5 1. A method of making a mixture of VNTR alleles and their flanking regions of the genomic DNA of one or more members of a species of interest, which method comprises the steps of:
- a) dividing genomic DNA of the species of interest into fragments,
- 10 b) ligating to each end of each fragment an adaptor thereby forming a mixture of adaptor-terminated fragments in which each 3'-end is blocked to prevent enzymatic chain extension,
- c) using a portion of the mixture of adaptor-terminated fragments as templates with an adaptor primer and a VNTR primer to
- 15 create a mixture of 5'-flanking VNTR amplimers,
- d) using a portion of the mixture of adaptor-terminated fragments as templates with an adaptor primer and a VNTR antisense primer to create a mixture of 3'-flanking VNTR amplimers,
- e) and using genomic DNA of the one or more members of the
- 20 species of interest as template with the mixture of 5'-flanking VNTR amplimers and/or the mixture of 3'-flanking VNTR amplimers as primers to make the desired mixture of VNTR alleles and their flanking regions.
2. The method of claim 1, wherein step b) is performed by terminating each 3'-end of each fragment to prevent enzymatic chain
- 25 extension, and ligating each 5'-end of each fragment to an adaptor, thereby forming a mixture of adaptor terminated fragments.
3. The method of claim 1 or claim 2, wherein in step c) the VNTR repeat sequences are removed from the 5'- flanking VNTR amplimers, and in step d) the VNTR repeat sequences are removed from
- 30 the 3'- flanking VNTR amplimers.

OFFICIAL RECORD

SUB  
B2SUB  
B1SUB  
A1

4. The method of any one of claims 1 to 3, wherein in step c) and/or d) the adaptor or primer used contains at least one phosphorothioate bond.

5. The method of any one of claims 1 to 4, wherein step e) is performed using as primers, either successively or together, both the mixture of 5'- flanking VNTR amplimers and the mixture of 3'- flanking VNTR amplimers.

6. The method of any one of claims 1 to 5, wherein there is used in step e) genomic DNA of one or more members of the species of interest which manifest a trait of interest, whereby the resulting mixture of VNTR alleles and their flanking sequences is representative of those which manifest the trait of interest

7. The method of claim 6 wherein in a step f) the strands of the mixture of VNTR alleles and their flanking regions are separated and then re-annealed and any mis-matches are separated and discarded.

8. The method of claim 7, wherein step f) is repeated to recover a single VNTR allele and its flanking regions.

9. The method of any one of claims 6 to 8, wherein at least one VNTR allele and its flanking sequences representative of those which manifest the trait of interest, is hybridised with a mixture of VNTR alleles and their flanking sequences representative of those which do not manifest the trait of interest, and at least one match and/or at least one mis-match is selected to provide at least one VNTR allele or fragment thereof which is characteristic of the trait of interest.

10. The method of claim 9, wherein the at least one VNTR allele and its flanking sequences representative of those which manifest the trait of interest, is provided with 3'- overlapping ends.

11. A portion of genomic DNA of one or more members of a species of interest, said portion consisting essentially of a representative mixture of alleles of a chosen VNTR sequence and their flanking regions on both sides.

SUB B3

12. The portion as claimed in claim 11, wherein the mixture of alleles is representative of those which manifest a trait of interest.

Sub a3

13. The portion as claimed in claim 11 or claim 12, wherein each member of the mixture has an adaptor at each of its 3'-end and its 5'-end.

SUB B5

14. A portion of genomic DNA of one or more members of a species of interest, said portion consisting essentially of a single VNTR allele and its flanking regions and an adaptor at each of its 3'-end and its 5'-end, said allele being characteristic of those which manifest a trait of interest.

15. A portion of genomic DNA of a species of interest, said portion consisting essentially of a representative mixture of 3'-flanking regions of a chosen VNTR sequence, each member of the mixture carrying an adaptor at its 3'-end, and a representative mixture of 5'-flanking regions of a chosen VNTR sequence, each member of the mixture carrying an adaptor at its 5'-end.

Sub a4

16. A method of treating nucleic acids which consist essentially of a mixture of polymorphic alleles, the mixture being representative of those which manifest a trait of interest, which method comprises separating and then re-annealing strands of the mixture, and separating and discarding any mis-matches.

SUB B1

17. The method of claim 16, wherein the mixture of polymorphic alleles is a mixture of alleles of a chosen VNTR sequence and their flanking regions.

SUB B1

18. The method of claim 17, wherein the method is repeated to recover a single VNTR allele and its flanking regions.

SUB B1

19. The method of any one of claims 16 to 18, wherein at least one VNTR allele and its flanking sequence representative of those which manifest the trait of interest, is hybridised with a mixture of VNTR alleles and their flanking sequences representative of those which do not manifest the trait of interest, and at least one match and/or at least one mis-match is selected to provide at least one VNTR allele or fragment thereof which is

Sub a5

characteristic of the trait of interest.

20. The method of claim 19, wherein the at least one VNTR allele and its flanking sequence representative of those which manifest the trait of interest, is provided with 3'-overlapping ends.

5 21. A method of making a mixture of amplimers which method comprises the steps of:

a) dividing genomic DNA of one or more members of a species of interest into fragments

b) ligating to each end of each fragment an adaptor thereby  
10 forming a mixture of adaptor-terminated fragments in which each 3'-end is blocked to prevent enzymatic chain extension, and

c) using a portion of the mixture of adaptor-terminated fragments as templates with an adaptor primer and a VNTR primer to create a mixture of 5'-flanking VNTR amplimers, and/or

15 d) using a portion of the mixture of adaptor-terminated fragments as templates with an adaptor primer and a VNTR antisense primer to create a mixture of 3'-flanking VNTR amplimers.

22. A method of identifying an allele which is linked to a trait of interest, which method comprises incubating together under hybridisation conditions: at least one molecule of nucleic acid containing a polymorphic allele and its flanking sequences representative of those which manifest the trait of interest; and a mixture of molecules of nucleic acid which contain polymorphic alleles and their flanking sequences representative of those which do not manifest the trait of interest; and selecting at least one match and/or at least one mis-match to provide at least one allele or fragment  
20 thereof which is linked to the trait of interest.

23. The method of claim 22, wherein the alleles are VNTR alleles.

24. The method of claim 22 or claim 23, wherein the at least one allele and its flanking sequences representative of those which manifest the trait of interest, is provided with 3'-overlapping ends.

SUB  
B<sup>9</sup>

25. Use of the portion of genomic DNA as claimed in claim 14 in a diagnostic assay.

26. The method of any one of claims 1 to 10 or 16 to 20, wherein the VNTR allele and its flanking regions, or the mixture of VNTR alleles and their flanking regions, is analysed by being applied under hybridisation conditions to an array of immobilised VNTR alleles and/or their flanking regions.

SUB  
A<sup>7</sup>

27. A kit comprising protocols and reagents for performing the method of any one of claims 1 to 10, 16 to 24 or 26.

add B<sup>10</sup>

add

add B<sup>10</sup>

## APPENDIX A

1. A method of making a mixture of VNTR alleles and their flanking regions of the genomic DNA of one or more members of a species of interest, which method comprises the steps of:

- a) dividing genomic DNA of the species of interest into fragments,
- b) ligating to each end of each fragment an adaptor thereby forming a mixture of adaptor-terminated fragments in which each 3'-end is blocked to prevent enzymatic chain extension,
- c) using a portion of the mixture of adaptor-terminated fragments as templates with an adaptor primer and a VNTR primer to create a mixture of 5'-flanking VNTR amplimers,
- d) using a portion of the mixture of adaptor-terminated fragments as templates with an adaptor primer and a VNTR antisense primer to create a mixture of 3'-flanking VNTR amplimers,
- e) and using genomic DNA of the one of more members of the species of interest as template with the mixture of 5'-flanking VNTR amplimers and/or the mixture of 3'-flanking VNTR amplimers as primers to make the desired mixture of VNTR alleles and their flanking regions.

2. The method of claim 1, wherein step b) is performed by terminating each 3'-end of each fragment to prevent enzymatic chain extension, and ligating each 5'-end of each fragment to an adaptor, thereby forming a mixture of adaptor terminated fragments.

3. The method of claim 1, wherein in step c) the VNTR repeat sequences are removed from the 5'-flanking VNTR amplimers, and in step d) the VNTR repeat sequences are removed from the 3'-flanking VNTR amplimers.

4. The method of claim 1, wherein in step c) and/or d) the adaptor or primer used contains at least one phosphorothioate bond.

5. The method of claim 1, wherein step e) is performed using as primers, either successively or together, both the mixture of 5'-flanking VNTR amplimers and the mixture of 3'-flanking VNTR amplimers.

6. The method of claim 1, wherein there is used in step e) genomic DNA of one or more members of the species of interest which manifest a trait of interest, whereby the resulting mixture of VNTR alleles and their flanking sequences is representative of those which manifest the trait of interest.

7. The method of claim 6 wherein in a step f) the strands of the mixture of VNTR alleles and their flanking regions are separated and then re-annealed and any mis-matches are separated and discarded.

8. The method of claim 7, wherein step f) is repeated to recover a single VNTR allele and its flanking regions.

9. The method of claim 6, wherein at least one VNTR allele and its flanking sequences representative of those which manifest the trait of interest, is hybridised with a mixture of VNTR alleles and their flanking sequences representative of those which do not manifest the

trait of interest, and at least one match and/or at least one mis-match is selected to provide at least one VNTR allele or fragment thereof which is characteristic of the trait of interest.

10. The method of claim 9, wherein the at least one VNTR allele and its flanking sequences representative of those which manifest the trait of interest, is provided with 3'-overlapping ends.

11. A portion of genomic DNA of one or more members of a species of interest, said portion consisting essentially of a representative mixture of alleles of a chosen VNTR sequence and their flanking regions on both sides.

12. The portion as claimed in claim 11, wherein the mixture of alleles is representative of those which manifest a trait of interest.

13. The portion as claimed in claim 11, wherein each member of the mixture has an adaptor at each of its 3'-end and its 5'-end.

14. A portion of genomic DNA of one or more members of a species of interest, said portion consisting essentially of a single VNTR allele and its flanking regions and an adaptor at each of its 3'-end and its 5'-end, said allele being characteristic of those which manifest a trait of interest.

15. A portion of genomic DNA of a species of interest, said portion consisting essentially of a representative mixture of 3'-flanking regions of a chosen VNTR sequence, each member of the mixture carrying an adaptor at its 3'-end, and a representative mixture of 5'-flanking regions of a chosen VNTR sequence, each member of the mixture carrying the same adaptor at its 5'-end.

16. A method of treating nucleic acids which consist essentially of a mixture of polymorphic alleles, the mixture being representative of those which manifest a trait of interest, which method comprises separating and then re-annealing strands of the mixture, and separating and discarding any mis-matches.

17. The method of claim 16, wherein the mixture of polymorphic alleles is a mixture of alleles of a chosen VNTR sequence and their flanking regions.

18. The method of claim 17, wherein the method is repeated to recover a single VNTR allele and its flanking regions.

19. The method of claim 16, wherein at least one VNTR allele and its flanking sequence representative of those which manifest the trait of interest, is hybridised with a mixture of VNTR alleles and their flanking sequences representative of those which do not manifest the trait of interest, and at least one match and/or at least one mis-match is selected to provide at least one VNTR allele or fragment thereof which is characteristic of the trait of interest.

20. The method of claim 19, wherein the at least one VNTR allele and its flanking sequence representative of those which manifest the trait of interest, is provided with 3'-overlapping ends.

21. A method of making a mixture of amplimers which method comprises the steps of:

- a) dividing genomic DNA of one or more members of a species of interest into fragments,
- b) ligating to each end of each fragment an adaptor thereby forming a mixture of adaptor-terminated fragments in which each 3'-end is blocked to prevent enzymatic chain extension, and
- c) using a portion of the mixture of adaptor-terminated fragments as templates with an adaptor primer and a VNTR primer to create a mixture of 5'-flanking VNTR amplimers, and/or
- d) using a portion of the mixture of adaptor-terminated fragments as templates with an adaptor primer and a VNTR antisense primer to create a mixture of 3'-flanking VNTR amplimers.

22. A method of identifying an allele which is linked to a trait of interest, which method comprises incubating together under hybridisation conditions: at least one molecule of nucleic acid containing a polymorphic allele and its flanking sequences representative of those which manifest the trait of interest; and a mixture of molecules of nucleic acid which contain polymorphic alleles and their flanking sequences representative of those which do not manifest the trait of interest; and selecting at least one match and/or at least one mis-match to provide at least one allele or fragment thereof which is linked to the trait of interest.

23. The method of claim 22, wherein the alleles are VNTR alleles.

24. The method of claim 22, wherein the at least one allele and its flanking sequences representative of those which manifest the trait of interest, is provided with 3'-overlapping ends.

25. Use of the portion of genomic DNA as claimed in claim 14 in a diagnostic assay.

26. The method of claim 1 or claim 16, wherein the VNTR allele and its flanking regions, or the mixture of VNTR alleles and their flanking regions, is analysed by being applied under hybridisation conditions to an array of immobilised VNTR alleles and/or their flanking regions.

27. A kit comprising protocols and reagents for performing the method of claim 1 or claim 16 or claim 24.